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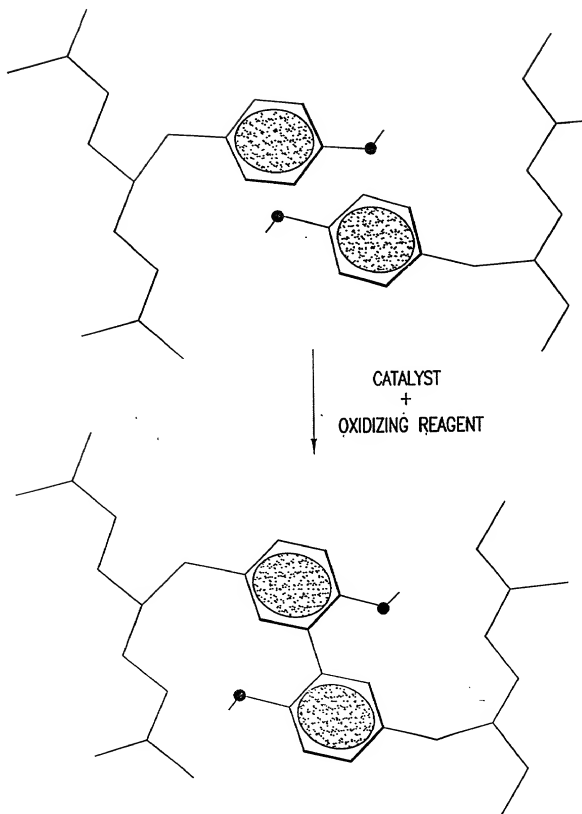


FIG.1A

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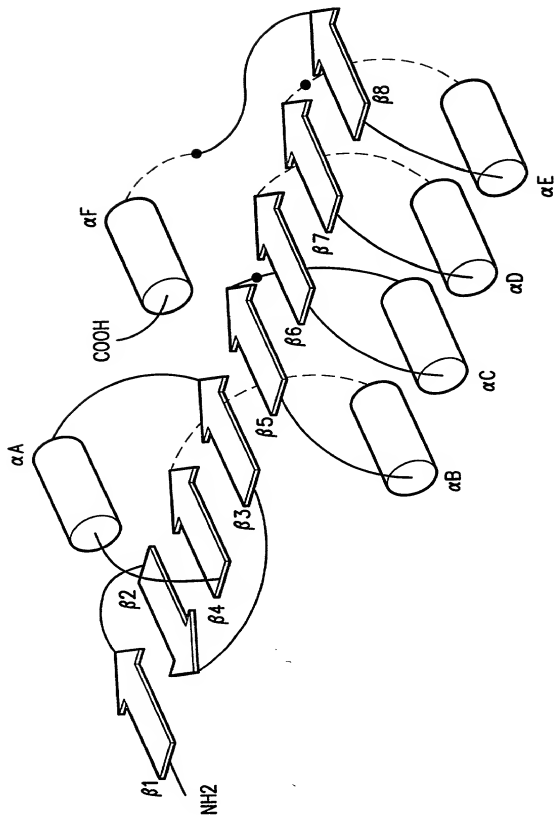


FIG.1B

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FIG. 1D

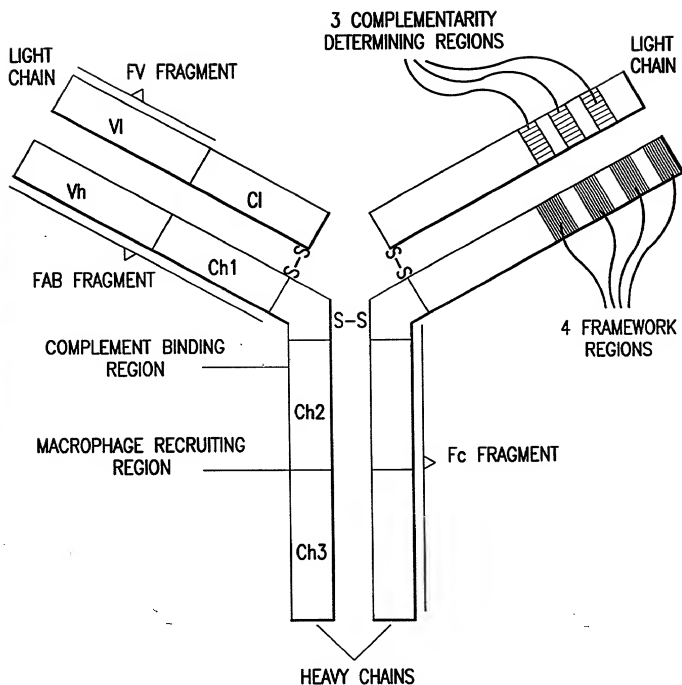


FIG.1D

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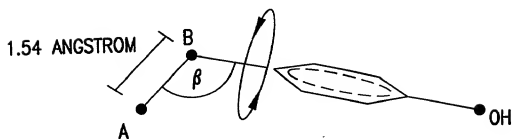


FIG.2A

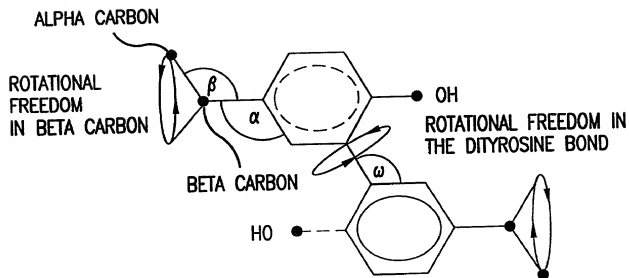


FIG.2B

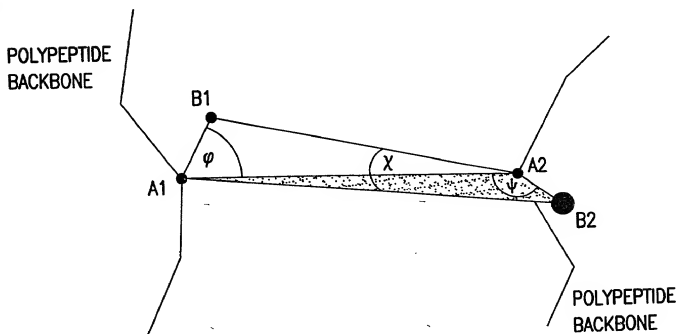


FIG.2C

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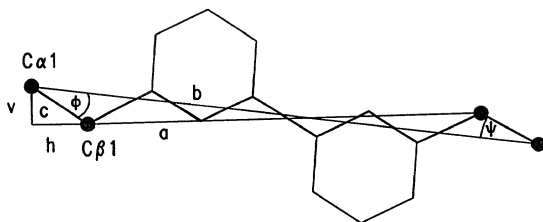


FIG.3A

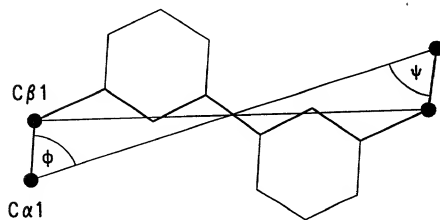


FIG.3B

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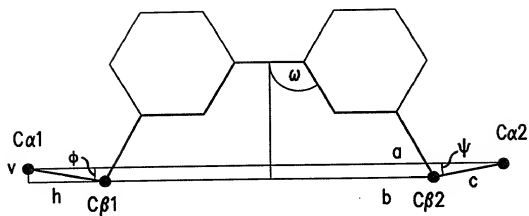


FIG. 4A

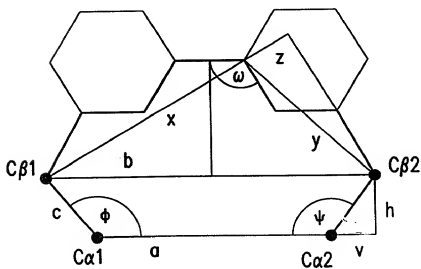


FIG. 4B

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LIGHT CHAIN (L)

CHAIN	K&W	ATOM	AMINO ACID	x COORDINATE	y COORDINATE	z COORDINATE
L	1	N	ASP	2.37	-5.00	-27.24
L	1	C α	ASP	2.98	-3.78	-26.64
L	1	C	ASP	1.91	-2.70	-26.52
L	1	O	ASP	1.33	-2.29	-27.53
L	1	C β	ASP	4.14	-3.29	-27.53
L	1	C γ	ASP	5.18	-2.49	-26.76
L	1	O δ 1	ASP	4.86	-1.38	-26.27
L	1	O δ 2	ASP	6.34	-2.97	-26.65
L	2	N	ILE	1.63	-2.26	-25.30
L	2	C α	ILE	0.60	-1.24	-25.07
L	2	C	ILE	1.19	0.15	-24.94
L	2	O	ILE	2.14	0.35	-24.94
L	2	C β	ILE	-0.21	-1.52	-23.78
L	2	C γ 1	ILE	-0.90	-2.88	-23.86
L	2	C γ 2	ILE	-1.24	-0.43	-23.58
L	2	C δ 1	ILE	-1.66	-3.26	-22.59
L	3
.
.

FIG.5A

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HEAVY CHAIN (H)

CHAIN	K&W	ATOM	AMINO ACID	x COORDINATE	y COORDINATE	z COORDINATE
H	1	N	GLU	11.12	-2.19	9.00
H	1	C α	GLU	11.43	-1.08	8.05
H	1	C	GLU	11.93	-1.63	6.71
H	1	O	GLU	13.10	-1.98	6.56
H	1	C β	GLU	12.47	-0.12	8.66
H	1	C γ	GLU	13.82	-0.75	9.05
H	1	C δ	GLU	13.70	-1.77	10.17
H	1	O ϵ 1	GLU	13.38	-1.36	11.31
H	1	O ϵ 2	GLU	13.94	-2.97	9.92
H	2	N	ILE	11.02	-1.70	5.74
H	2	C α	ILE	11.36	-2.24	4.42
H	2	C	ILE	12.10	-1.22	3.59
H	2	O	ILE	11.77	-0.04	3.64
H	2	C β	ILE	10.11	-2.68	3.62
H	2	C γ 1	ILE	9.31	-3.73	4.39
H	2	C γ 2	ILE	10.52	-3.22	2.28
H	3	C δ 1	ILE	8.49	-3.17	5.55
H	3
.
.

FIG.5B

000725.041001

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Fv FRAGMENT 1

							Ch K&W	L 1	L 2	L 3	L 4	L 5
							At	Cα	Cα	Cα	Cα	Cα
							AA	Asp	Ile	.	.	.
							x	2.98	0.60	.	.	.
							y	-3.78	-1.24	.	.	.
							z	-26.64	-25.07	.	.	.
Ch	K&W	At	AA	x	y	z						
H	1	Cα	Glu	11.43	-1.08	8.05	35.80	34.84
H	2	Cα	Ile	11.36	-2.24	4.42	32.21	31.42
H	3	Cα
H	4	Cα
H	5	Cα

FIG.6A

Fv FRAGMENT 2

							Ch K&W	L 1	L 2	L 3	L 4	L 5
							At	Cα	Cα	Cα	Cα	Cα
							AA	Glu	Ser	.	.	.
							x	35.61	31.94	.	.	.
							y	83.10	83.89	.	.	.
							z	56.99	56.85	.	.	.
Ch	K&W	At	AA	x	y	z						
H	1	Cα	Glu	10.23	61.09	64.74	34.48	32.46
H	2	Cα	Val	13.63	62.72	65.19	31.07	29.20
H	3	Cα
H	4	Cα
H	5	Cα

FIG.6B

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Fv FRAGMENT 3

Ch	K&W	At	AA	x	y	z	Ch K&W	L 1	L 2	L 3	L 4	L 5
							At	C α	C α	C α	C α	C α
							AA	Glu	Ser	.	.	.
				x			x	19.56	19.09	.	.	.
				y			y	-13.02	-15.06	.	.	.
				z			z	-15.86	-12.67	.	.	.
H	1	C α	GLN	26.71	9.76	10.88		35.84	35.05	.	.	.
H	2	C α	Val	27.45	8.61	7.34		32.69	32.11	.	.	.
H	3	C α
H	4	C α
H	5	C α

FIG.6C

FIG. 7A

RESIDUE	PAIRS	AVERAGE	ST.DEV.	MAX	MIN	MEDIAN
H1	L1	35.38	0.78	35.84	34.48	35.80
H1	L2	34.12	1.44	35.05	32.46	34.84
H1	L3
H1	L4
.
.
H1	L106
H2	L1	31.99	0.83	32.69	31.07	32.21
H2	L2	30.91	1.52	32.11	29.20	31.41
H2	L3
H2	L4
.
.
H2	L106
H3	L1

FIG. 7A

RESIDUE	PAIRS	AVERAGE	ST.DEV.	MAX	MIN	MEDIAN
H1	L1	35.09	1.56	37.37	31.23	35.54
H1	L2	34.00	1.87	37.36	29.92	34.38
H1	L3
H1	L4
.
.
H1	L106
H2	L1	32.26	1.57	36.71	30.34	32.14
H2	L2	31.32	1.99	36.77	29.20	31.11
H2	L3
H2	L4
.
.
H2	L106
H3	L1

FIG. 7B

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							Ch	L	L	L	L	L
							K&W	1	2	3	4	5
							At	C β	C β	C β	C β	C β
							AA	ASP	ILE	.	.	.
							x	4.14	-0.21	.	.	.
							y	-3.29	-1.52	.	.	.
							z	-27.53	-23.78	.	.	.
Ch	K&W	At	AA	x	y	z						
H	1	C β	GLU	12.47	-0.12	8.66		37.27	34.85	.	.	.
H	2	C β	ILE	10.11	-2.68	3.62		31.73	29.30	.	.	.
H	3	C β
H	4	C β
H	5	C β

FIG.8

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ALPHA DISTANCES							Ch	L	L	L	L	L
							K&W	1	2	3	4	5
							At	C α	C α	C α	C α	C α
							AA	ASP	ILE	.	.	.
							x	2.98	0.60	.	.	.
							y	-3.78	-1.24	.	.	.
							z	-26.64	-25.07	.	.	.
Ch	K&W	At	AA	x	y	z						
H	1	C α	GLU	11.43	-1.08	8.05		35.80	34.84	.	.	.
H	2	C α	ILE	11.36	-2.24	4.42		32.21	31.42	.	.	.
H	3	C α
H	4	C α
H	5	C α

FIG.9A

BETA DISTANCES							Ch	L	L	L	L	L
							K&W	1	2	3	4	5
							At	C β	C β	C β	C β	C β
							AA	ASP	ILE	.	.	.
							x	4.14	-0.21	.	.	.
							y	-3.29	-1.52	.	.	.
							z	-27.53	-23.78	.	.	.
Ch	K&W	At	AA	x	y	z						
H	1	C β	GLU	12.47	-0.12	8.66		37.27	34.85	.	.	.
H	2	C β	ILE	10.11	-2.68	3.62		31.73	29.30	.	.	.
H	3	C β
H	4	C β
H	5	C β

FIG.9B

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DIFFERENCE			Ch	L	L	L	L	L
			K&W	1	2	3	4	5
DIFFERENCES BETWEEN RESIDUE PAIR ALPHA- AND BETA CARBON DISTANCES			AA	ASP	ILE	.	.	.
						.	.	.
						.	.	.
						.	.	.
Ch	K&W	AA						
H	1	GLU	-1.47 -0.01 . . .					
H	2	ILE	0.48 2.10 . . .					
H	3
H	4
H	5

FIG.9C

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		L1	L2	L3	L4	L5	.	.
Fv FRAGMENT 1	H1	-1.47	-0.01
	H2	0.48	2.10
	H3
	H4

	.	L1	L2	L3	L4	L5	.	.
Fv FRAGMENT 2	H1	-1.61	0.46
	H2	0.18	2.04
	H3
	H3

	.	L1	L2	L3	L4	L5	.	.
Fv FRAGMENT 3	H1	0.92	1.59
	H2	0.69	1.31
	H3
	H3

	.	L1	L2	L3	L4	L5	.	.
Fv FRAGMENT 4	H1

FIG.10

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RESIDUE PAIRS		AVERAGE	Strd.DEV.	MAX	MIN	MEDIAN
H1	L1	-0.72	1.42	0.92	-1.61	-1.47
H1	L2	0.68	0.82	1.59	-0.01	0.46
H1	L3
H1	L4
.
.
H1	L106
H2	L1	0.45	0.26	0.69	0.18	0.48
H2	L2	0.68	0.82	1.59	-0.01	0.46
H2	L3
H2	L4
.
.
H2	L106
H3	L1

FIG.11A

RESIDUE PAIRS		AVERAGE	Strd.DEV.	MAX	MIN	MEDIAN
H1	L1	-0.68	1.04	0.92	-2.20	0.83
H1	L2	0.34	0.82	2.37	-0.54	0.09
H1	L3
H1	L4
.
.
H1	L106
H2	L1	0.74	0.69	1.83	-0.18	0.59
H2	L2	1.78	0.50	2.55	0.75	1.94
H2	L3
H2	L4
.
.
H2	L106
H3	L1

FIG.11B

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Res.	AA	F	AA	F	AA	F	AA	F	AA	F	AA	F
1	Glu	58	Glu	24	Asp	3	Glu	3	Gly	2	Ala	1
2	Val	99	Ile	2	Ala	1	Glu	1	Met	1	-	-
3	Gln	90	Thr	5	Glu	3	His	2	Leu	2	Lys	2
4	Leu	101	Val	3	-	-	-	-	-	-	-	-

FIG.12A

Amino Acid	van der Waals volumes [Å ³]	Hydrophobicity
Ala	67	0.62
Arg	148	-2.50
Asn	96	-0.78
Asp	91	-0.90
Cys	86	0.29
Gln	114	-0.85
Glu	109	-0.79
Gly	48	0.30
His	118	-0.40
Ile	124	1.40
Leu	124	1.10
Lys	135	-1.50
Met	124	0.64
Phe	135	1.20
Pro	90	0.12
Ser	73	-0.18
Thr	93	-0.05
Trp	163	0.81
Tyr	141	0.26
Val	105	1.10

FIG.12B

Res.	AA	F	AA	F	AA	F	AA	F	AA	F	AA	F
1	109	61	109	24	91	3	48	2	67	1	-	-
2	105	99	124	2	67	1	109	1	124	1	-	-
3	114	90	93	5	109	3	118	2	124	2	135	2
4	124	101	105	3	-	-	-	-	-	-	-	-

FIG.12C

108140-55242880

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VAN DER WAALS VOLUMES

CHAIN	K&W	CONS.	AVERAGE	WEIGHTED StDev.	AVERAGE	UNWEIGHTED StDev.
H	1	Glu	108	11	90	27
H	2	Val	105	5	106	23
H	3	Gln	114	6	116	14
H	4	Leu	123	3	115	13

FIG.13A

HYDROPHOBICITY

CHAIN	K&W	CONS.	AVERAGE	WEIGHTED StDev.	AVERAGE	UNWEIGHTED StDev.
H	1	Glu	-0.77	0.24	-0.37	0.72
H	2	Val	1.08	0.20	0.59	0.84
H	3	Gln	-0.78	0.33	-0.42	0.89
H	4	Leu	1.10	0.00	1.10	0.00

FIG.13B

FOOTNOTES

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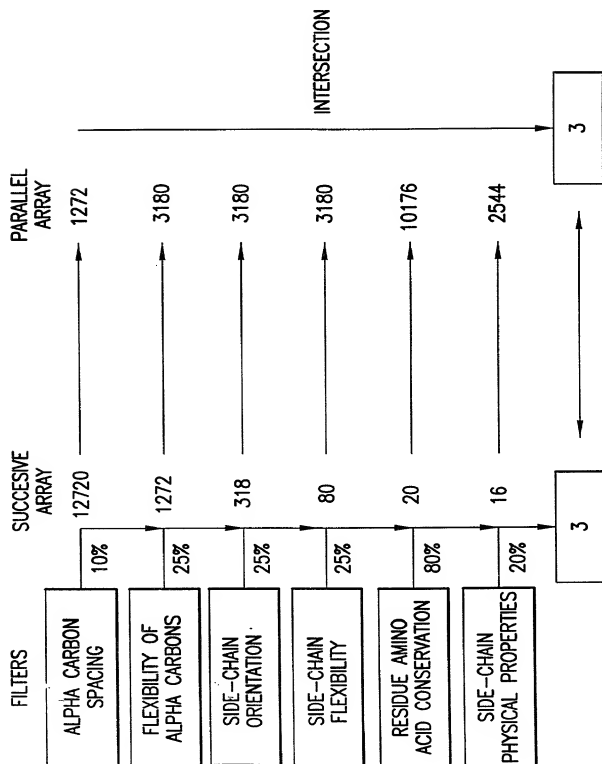


FIG.14

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C. Antarctica Lipase B Nucleotide and Amino Acid Sequence

10 20 30 40 50 60
 ctaccttcgggttcggaccctgccttttcgcagcccaagtcggtgctcgatgcgggtctg
 L P S G S D P A F S Q P K S V L D A G L
 70 80 90 100 110 120
 acctgccagggtgcttcgccatcctcggtctccaaaccatcctctcgtcccggaacc
 T C Q G A S P S S V S K P I L L V P G T
 130 140 150 160 170 180
 ggcaccacaggtccacagtcgttcgactcgaactggatccccctctcaacgcagttgggt
 G T T G P Q S F D S N W I P L S T Q L G
 190 200 210 220 230 240
 tacacacctgctggatctcaccctccgcggttcgctcaacgcacccaggtcaacacg
 Y T P C W I S P P P F M L N D T Q V N T
 250 260 270 280 290 300
 gagtacatggtcaacgcgcattcaccgcgtctacgctgggtcgggcaacaacagcttccc
 E Y M V N A I T A L Y A G S G N N K L P
 310 320 330 340 350 360
 gtgcttacctggtcccagggtggtctggttgacacagtggggtctgaccttcttcccagt
 V L T W S Q G G L V A Q W G L T F F P S
 370 380 390 400 410 420
 atcaggtccaaggtcgatcgacttatggcctttgcgcccgaactacaagggcaccgctcctc
 I R S K V D R L M A F A P D Y K G T V L
 430 440 450 460 470 480
 gcgcgacctctcgatgcactcgcggttagtgcaacctcgtatggcagcaaacaccggt
 A G P L D A L A V S A P S V W Q Q T T G
 490 500 510 520 530 540
 tcggcactcaccaccgcactccgaaacgcaggtggtgtgacccagatcgtgccaccacc
 S A L T T A L R N A G G L T Q I V P T T
 550 560 570 580 590 600
 aacctctactcggcgaccgacgagatcggttcagcctcaggtgtccaactcgccactcgac
 N L Y S A T D E I V Q P Q V S N S P L D
 610 620 630 640 650 660
 tcatcctacctcttcaacggaagaacggttcaggcacagccggtgtgtggcgccgtgttc
 S S Y L F N G K N V Q A Q A V C G P L F
 670 680 690 700 710 720
 gtcatcgaccatgcaggctcgctcacctcgagttctcctacgtcgtcggtcgatccgcc
 V I D H A G S L T S Q F S Y V V G R S A
 730 740 750 760 770 780
 ctgcgctccaccacgggagcgtcgtagtgacactatggcattacggactgcaacctc
 L R S T T G Q A R S A D Y G I T D C N P
 790 800 810 820 830 840
 cttcccgcaatgatctgactcccgagcaaaagggtcgccggtgctcgctcctggcgccg
 L P A N D L T P E Q K V A A A A L L A P
 850 860 870 880 890 900
 gcagctgcagccatcggtggcggtccaagcagaactgcgagcccgacctcatgccctac
 A A A A I V A G P K Q N C E P D L M P Y
 910 920 930 940 950
 gccgcgccctttgcagtaggcaaaaggacctgctccggcatcgctcaccctctga
 A R P F A V G K R T C S G I V T P *

FIG. 15A

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PCR Oligos for *Candida antarctica* Lipase B**Oligos for pPal-CALB**

Primer A: 5'atg gga att oca tca tca tca tca cag cag cgg cct acc ttc cgg ttc gga ccc3'

Primer B: 5'ctc ttg gcg gcc gcc tat cag ggg gtg acg atg ccg g3'

Oligos for Point Mutations (made in pPal-CALB)**M1- F9Y**

Primer M1F: 5'atg gga att oca tca tca tca tca cag cag cgg cct acc ttc cgg ttc gga ccc tgc ctA ttc gc3'

M2- W52Y

Primer M2F: 5'cga ctc gaa ctA Cat ccc cct ctc3'

Primer M2R: 5'gag agg ggg atG Tag ttc gag tcg3'

M3- F117Y

Primer M3F: 5'ggg tctg acc tAc ttc ccc agt atc3'

Primer M3R: 5'gat act ggg gaa gTa ggt cag acc c3'

Oligos for pYal-CALB

Primer C:

5'-cgA Tga gat ttc ctt caa ttt -3'

Primer D:

5'-5'tct aga aag gtg gcg gcc gcc -3'

Oligos for error-prone PCR

Primer E:

5'gaa gct gga ttc cat cat cat c3'

Primer D:

5'-5'tct aga aag gtg gcg gcc gcc -3'

FIG. 15B

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Subtilisin E Nucleotide and Amino Acid Sequence

10 20 30 40 50 60 70 80
 atgtctgtgcagggtgcgggaaaaagcagttacagaaaaagaatacatgttcggatttaaacagacaaatgagtgccatgag
 M S V Q A A G K S S T E K K Y I V G F K Q T M S A M S
 90 100 110 120 130 140 150 160
 ttccgccaagaaaaaggtgttatttctgaaaaagcggaaggttcaaaagcaatttaagtattgttaacgcggcgcgag
 S A K K K D V I S S K G G K V Q K Q F K Y V N A A A
 170 180 190 200 210 220 230 240
 caacattggatgaaaaagctgttaaaagaattgaaaaagatccgagcgttgcatatgtggaagaagatcatattgcacat
 A T L D E K A V K E L K K D P S V A Y V E E D H I A H
 250 260 270 280 290 300 310 320
 gaatatgcgcaatctgttcttattgtgcatttctcaaatataagcgcggctcttcaactctcaaggctacacaggctcttaa
 E Y A Q S V P Y G I S Q I K A P A L H S Q G Y T G S N
 330 340 350 360 370 380 390 400
 cgtaaaaagtagctgttatgcacagcgggaattgactcttctcaactcgtgacttaaacgtcagaggcggagcaagcttggtac
 V K V A V I D S G I D S S H P D L N V R G G A S F V
 410 420 430 440 450 460 470 480
 cttctgaaacaaacccataccaggacggcagttctcaacggtaacgcatgtagccggtagcattgccgtctttaataactca
 P S E T N P Y Q D G S S H G T H V A G T I A A L N N S
 490 500 510 520 530 540 550 560
 atcgggtgttctggcggttagcccaagcgcatttattatgcagtaaaagtgcttgatcaacaggaagcggccaatatag
 I G V L G V S P S A S L Y A V K V L D S T G S G Q Y S
 570 580 590 600 610 620 630 640
 ctggattattacgcgcattgagtgggccatttccaacaattatggatgttatcaacatgagccttggcggcactcgtggtt
 W I I N G I E W A I S N N M D V I N M S L G G P T G
 650 660 670 680 690 700 710 720
 ctacagcgtgaaaaacagtcgttgacaaaagcgtttccagcgggtatcgtcgttgctgcgcgacgcggaaacgaaggttca
 S T A L K T V V D K A V S S G I V V A A A A G N E G S
 730 740 750 760 770 780 790 800
 tcoggaagcacaagcacagtcgggtacccctgcaaaaatctcttactattgcagtaggtgcggtaaacagcagcacaacca
 S G S T S T V G Y P A K Y P S T I A V G A V N S S N Q
 810 820 830 840 850 860 870 880
 aagagcttcattctccagcgcaggttctgagcttgatgtgatggctcctggcgtgtccatccaagaacacttctcggag
 R A S F S A G S E L D V M A P G V S I Q S T L P G
 890 900 910 920 930 940 950 960
 gcacttacggcgttataacgggaagctccatggcgactctcactgttcggagcagcagcgttaattctttctaagcac
 G T Y G A Y N G T S M A T P H V A G A A A L I L S K H
 970 980 990 1000 1010 1020 1030 1040
 cpgacttggacaacacgcgaagtcocgtgatcgttttagaaagcactgcaacatatcttggaaactcttctactatggaaa
 P A T W T N A Q V R D R L E S T A T Y L G N S F Y Y G K
 1050 1060 1070
 agggttaatcaacgtacaaagcagctgcacataa
 G L I N V Q A A A Q *

FIG. 16A

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Subtilisin Amino Acid Alignment

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
ALA	GLN	SER	VAL	PRO	TRP	GLY	ILE	SER	ARG	VAL	GLN	ALA	PRO	ALA	ALA	HIS	ASN
ALA	GLN	SER	VAL	PRO	TYR	GLY	ILE	SER	GLN	ILE	LYS	ALA	PRO	ALA	LEU	HIS	SER
ALA	LYS	CYS	VAL	SER	TYR	GLY	VAL	SER	GLN	ILE	LYS	ALA	PRO	ALA	LEU	HIS	SER
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
ARG	GLY	LEU	THR	GLY	SER	GLY	VAL	LYS	VAL	ALA	VAL	LEU	ASP	THR	GLY	ILE	SER
GLN	GLY	TYR	THR	GLY	SER	ASN	VAL	LYS	VAL	ALA	VAL	ILE	ASP	SER	GLY	ILE	ASP
GLN	GLY	TYR	THR	GLY	SER	ASN	VAL	LYS	VAL	ALA	VAL	ILE	ASP	SER	GLY	ILE	ASP
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
THR	---	HIS	PRO	ASP	LEU	ASN	ILE	ARG	GLY	GLY	ALA	SER	PHE	VAL	PRO	SER	GLU
SER	SER	HIS	PRO	ASP	LEU	ASN	VAL	ARG	GLY	GLY	ALA	SER	PHE	VAL	PRO	SER	GLU
SER	SER	HIS	PRO	ASP	LEU	ASN	VAL	ALA	GLY	GLY	ALA	SER	PHE	VAL	PRO	SER	GLU
55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
---	---	PRO	SER	THR	GLN	ASP	GLY	ASN	GLY	HIS	GLY	THR	HIS	VAL	ALA	GLY	THR
THR	ASN	PRO	TYR	---	GLN	ASP	GLY	SER	SER	HIS	GLY	THR	HIS	VAL	ALA	GLY	THR
THR	ASN	PRO	PHE	---	GLN	ASP	ASN	ASN	SER	HIS	GLY	THR	HIS	VAL	ALA	GLY	THR
73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
ILE	ALA	ALA	LEU	ASN	ASN	SER	ILE	GLY	VAL	LEU	GLY	VAL	ALA	PRO	ASN	ALA	GLU
ILE	ALA	ALA	LEU	ASN	ASN	SER	ILE	GLY	VAL	LEU	GLY	VAL	SER	PRO	SER	ALA	SER
---	---	---	---	---	---	---	---	---	VAL	LEU	ALA	VAL	ALA	PRO	SER	ALA	SER
91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108
LEU	TYR	ALA	VAL	LYS	VAL	LEU	GLY	ALA	SER	GLY	SER	GLY	SER	VAL	SER	SER	ILE
LEU	TYR	ALA	VAL	LYS	VAL	LEU	ASP	SER	THR	GLY	SER	GLY	GLN	TYR	SER	TRP	ILE
LEU	TYR	ALA	VAL	LYS	VAL	LEU	GLY	ALA	ASP	GLY	SER	GLY	GLN	TYR	SER	TRP	ILE
109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126
ALA	GLN	GLY	LEU	GLU	TRP	ALA	GLY	ASN	ASN	GLY	MET	HIS	VAL	ALA	ASN	LEU	SER
ILE	ASN	GLY	ILE	GLU	TRP	ALA	ILE	SER	ASN	ASN	MET	ASP	VAL	ILE	ASN	MET	SER
ILE	ASN	GLY	ILE	GLU	TRP	ALA	ILE	ALA	ASN	ASN	MET	ASP	VAL	ILE	ASN	MET	SER
127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144
LEU	GLY	SER	PRO	SER	PRO	SER	ALA	THR	LEU	GLU	GLN	ALA	VAL	ASN	SER	ALA	THR
LEU	GLY	GLY	PRO	THR	GLY	SER	THR	ALA	LEU	LYS	THR	VAL	VAL	ASP	LYS	ALA	VAL
LEU	GLY	GLY	PRO	SER	GLY	SER	ALA	LEU	LYS	ALA	VAL	ASP	VAL	ASP	LYS	ALA	VAL
145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162
SER	ARG	GLY	VAL	LEU	VAL	VAL	ALA	ALA	ALA	SER	GLY	ASN	SER	GLY	---	ALA	GLY
SER	SER	GLY	ILE	VAL	VAL	ALA	ALA	ALA	ALA	ALA	GLY	ASN	GLU	GLY	SER	SER	GLY
ALA	SER	GLY	VAL	VAL	VAL	VAL	ALA	ALA	ALA	ALA	GLY	ASN	GLU	GLY	THR	SER	GLY

FIG. 16B

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Subtilisin Amino Acid Alignment (cont'd.)

163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
 ILE SER --- --- TYR PRO ALA ARG TYR ALA ASN ALA MET ALA VAL GLY ALA
 THR SER THR VAL GLY TYR PRO ALA LYS TYR PRO SER THR ILE ALA VAL GLY ALA
 SER SER THR VAL GLY TYR PRO GLY LYS TYR PRO SER VAL ILE ALA VAL GLY ALA

181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198
 THR ASP GLN ASN ASN ASN ARG ALA SER PHE SER GLN TYR GLY ALA GLY LEU ASP
 VAL ASN SER SER ASN GLN ARG ALA SER PHE SER SER ALA GLY SER GLU LEU ASP
 VAL ASP SER SER ASN GLN ARG ALA SER PHE SER SER VAL GLY PRO GLU LEU ASP

199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216
 ILE VAL ALA PRO GLY VAL ASN VAL GLN SER THR TYR PRO GLY SER THR TYR ALA
 VAL MET ALA PRO GLY VAL SER ILE GLN SER THR LEU PRO GLY GLY THR TYR GLY
 VAL MET ALA PRO GLY VAL SER ILE CYS SER THR LEU PRO GLY ASN LYS TYR GLY

217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234
 SER LEU ASN GLY THR SER MET ALA THR PRO HIS VAL ALA GLY ALA ALA ALA LEU
 ALA TYR ASN GLY THR CYS MET ALA THR PRO HIS VAL ALA GLY ALA ALA ALA LEU
 ALA LYS SER GLY THR SER MET ALA SER PRO HIS VAL ALA GLY ALA ALA ALA LEU

235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252
 VAL LYS GLN LYS ASN PRO SER TRP SER ASN VAL GLN ILE ARG ASN HIS LEU LYS
 ILE LEU SER LYS HIS PRO THR TRP THR ASN ALA GLN VAL ARG ASP ARG LEU GLU
 ILE LEU SER LYS HIS PRO ASN TRP THR ASN THR GLN VAL ARG SER SER LEU GLU

253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270
 ASN THR ALA THR SER LEU GLY SER THR ASN LEU TYR GLY SER GLY LEU VAL ASN
 SER THR ALA THR TYR LEU GLY ASN SER PHE TYR TYR GLY LYS GLY LEU ILE ASN
 ASN THR THR THR LYS LEU GLY ASN SER PHE TYR TYR GLY LYS GLY LEU ILE ASN

271 272 273 274 275 276
 ALA GLU ALA ALA THR ARG
 VAL GLN ALA ALA ALA GLN
 VAL GLN ALA ALA ALA GLN

- FIG. 16C

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PCR Oligos for Subtilisin E

A primer-

5'-ccg agc gttg cat atg tgg aag-3'

1- K27Y

F 5'-ggc tct aac gta TaT gta gct gtt atc-3'

R 5'-gat aac agc tac AtA tac gtt aga gcc-3'

2- K237Y

F 5'-tta att ctt tct TaC cac cgg act tgg-3'

R 5'-cca agt cgg gfg GtA aga aag aat taa c-3'

3.1- D36Y

F 5'-gac agc gga att T act ctt etc atc-3'

R 5'-gat gag aag agt A aat tcc gct gtc-3'

3.2- P210Y

F 5'-caa agc aca ctt TAt gga ggc act tac-3'

R 5'-ta agt gcc tcc aTA aag tgt gct ttg-3'

4.1- K170Y

F 5'-ggc tac cct gea TaT tat cct tct act a-3'

R 5'-agt aga agg ata AtA tgc agg gta gcc-3'

4.2- E195Y

F 5'-agc gca ggt tct TaT ctt gat gtg atg -3'

R 5'-cat cac atc aag AtA aga acc tgc gct-3'

B-primer-

5'-tta gga tcc tta atg atg atg atg atg ttg tgc
agc tgc tfg tac gtt gat-3'

5.1- G61Y

F 5'-cca tac cag gac TAc agt tct cac gg-3'

R 5'-cc gfg aga act gTA gtc ctg gta tgg-3'

5.2- S98Y

F 5'-aa gfg ctt gat TAT aca gga agc ggc-3'

R 5'-gcc gct tcc tgt ATA atc aag cac ti-3'

6.1- H17Y

F 5'-gcg cgg gct ctt TAc tct caa ggc t-3'

R 5'-a gcc ttg aga gTA aag agc cgg cgc-3'

6.2- P86Y

F 5'-ctg ggc gtt agc TAT agc gca tca tta-3'

R 3'-taa tga tgc gct ATA gct aac gcc cag-3'

7- P201Y

F 5'-gat gfg atg gct TAt ggc gfg tcc atc-3'

R 5'-gat gga cac gcc aTA agc cat cac atc-3'

FIG. 16D